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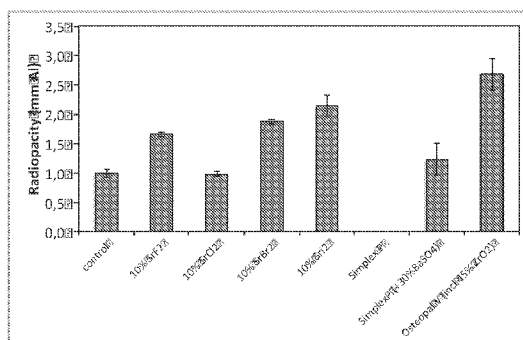


FIG. 2

(57) Abstract: Compositions suitable for use as, for example, dental, craniofacial or long-bone fillers, percutaneous vertebroplasty agents and kyphoplasty agents, comprise an injectable biomaterial cement and a radiopacity-improving agent in an amount sufficient to improve the radiopacity of the injectable biomaterial *in vivo*. The radiopacity agent comprises strontium bromide (SrBr₂) and/or strontium iodide (SrI₂).

COMPOSITIONS COMPRISING INJECTABLE BIOMATERIAL CEMENT AND RADIOPACITY-IMPROVING AGENT

FIELD OF THE INVENTION

[0001] The present invention is directed to cement compositions having good radiopaque properties in combination with other properties which are advantageous for use in vivo, such as biocompatibility, osteoconductivity, and/or resorbability in vivo. Specifically, the present invention is directed to such compositions wherein the radiopacity agent comprises a salt of strontium and a halogen, specifically, strontium bromide and/or strontium iodide.

BACKGROUND OF THE INVENTION

[0002] Injectable biomaterials are used in, for example, orthopedics, tissue engineering, and drug delivery, or as scaffolds for bone repair. Common injectable biomaterials include, but are not limited to, acrylic bone cements and calcium-based cements such as calcium phosphate cements (CPCs), calcium sulphate cements, and calcium silicate cements. Radiopacifying agents are typically included in injectable biomaterial formulations in order to improve radiopaque properties for radiography, computer tomography and/or real time fluoroscopy. Radiopacity refers to the relative difficulty for electromagnetic radiation, such as X-rays, to pass through a material. The radiopacity assists in, inter alia, proper delivery and positioning of injectable implants in the body and monitoring of injected implants over time.

[0003] Inorganic radiopaque agents are usually based on barium or zirconium. Acrylic bone cement formulations often contain a certain amount of an insoluble radiopacifying agent, typically barium sulphate (BaSO_4) or zirconium dioxide (ZrO_2). However, $\text{BaSO}_4/\text{ZrO}_2$ radiopaque agents are not soluble in water and may be detrimental to some mechanical and biological properties of the cements that contain them (Ginebra et al. 2002; Lazarus et al. 1994). When acrylic bone cements are used in, for example, endoprostheses fixation, the mobility of

the joints may cause particles of the radiopaque agents to be released into the joint contact area or into the body. Free $\text{BaSO}_4/\text{ZrO}_2$, especially ZrO_2 particles, can cause tribological damage of the contact in, e.g., hip-joint prostheses, and/or can generate more particles by abrasion (Cooper et al. 1991).

[0004] Additionally, as noted, the $\text{BaSO}_4/\text{ZrO}_2$ particles may be released into the body. These particles are neither water-soluble nor degradable and therefore are not assimilated through biological routes. Cell studies have shown that the presence of $\text{BaSO}_4/\text{ZrO}_2$ particles can cause macrophage differentiation into osteoclasts (Sabokbar et al 1997), thereby triggering bone resorption. The addition of $\text{BaSO}_4/\text{ZrO}_2$ is therefore disadvantageous in calcium phosphate cements (CPC) which are resorbable. Therefore, the addition of inert radiopacity agents is not compatible, i.e. CPCs resorb in vivo whereas the inert radiopacifying agents do not and disadvantageously lead to a non-resorbable part in the hardened cement end product.

[0005] The chemical design of first generation iodine-based organic radiopaque agents has been discussed in the literature (Archer 1959). Organic radiopaque agents can be ionic or non-ionic compounds with covalently bonded iodine. Examples of ionic organic compounds that are currently used as contrast agents are: diatrizoic acid, metrizoic acid, and ioxaglic acid. On the other hand, examples of non-ionic contrast agents that are used as contrast agents are: iopamidol, iohexol, ioxilan, iopromide, and iodixanol. Organic radiopaque agents are administered orally, by enema or intravenous and are typically used for visualization of blood vessels and organs, but their use is limited due to high cost and side effects.

[0006] Calcium phosphate cements are a family of biomaterials that exhibit excellent biocompatibility and osteoconductivity. They are typically composed of a powder component that contains one or more calcium orthophosphates that, depending on the specific composition,

precipitates in the form of hydroxyapatite or dicalcium phosphate dihydrate, upon mixing with an aqueous solution. However, the intrinsic radiopacity of the calcium phosphate cements is limited and typically the addition of a contrast agent is required for certain applications, for example, spinal applications. Although calcium phosphate cements approved for use in vertebroplasty are considered intrinsically radiopaque, their radiopacity is sometimes not enough and it can be troublesome to distinguish them from bone. However, the addition of $\text{BaSO}_4/\text{ZrO}_2$ is not recommended in calcium phosphate cements due to the noted negative effects on the mechanical properties and biocompatibility.

[0007] It has been reported that Sr-ions may stimulate osteoblasts and reduce the activity of osteoclasts, which would result in new bone formation (Marie et al. 2001). The literature also describes the use of strontium carbonate (SrCO_3), and strontium chloride (SrCl_2) in its hydrated form ($\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$), both as radiopaque agents and/or sustained release sources of Sr^{2+} (Wang et al. 2007; Tadier et al. 2011).

[0008] US 4,797,431; US 5,106,301; US 2003/0199605 A1; and US 2010/0068677 A1 describe the use of strontium fluoride (SrF_2) and strontium chloride as radiopaque agents, mostly for dental applications; however, SrF_2 has a very low solubility and reduces the mechanical properties of the hardened end product significantly.

[0009] Accordingly, injectable biomaterial cements with improved radiopacity are desired. Additionally, a resorbable radiopacifying agent that maintains the combined high mechanical strength and biocompatibility of a hardened CPC is highly desirable.

SUMMARY OF THE INVENTION

[0010] It is an object of the present invention to provide injectable biomaterial cement compositions having good radiopaque properties in combination with other properties which are

advantageous for use in vivo, such as biocompatibility, osteoconductivity, and/or resorbability in vivo.

[0011] In one embodiment, the invention is directed to compositions comprising an injectable biomaterial cement and a radiopacity-improving agent in an amount sufficient to improve the radiopacity of the injectable biomaterial cement in vivo, wherein the radiopacity agent comprises strontium bromide (SrBr_2) and/or strontium iodide (SrI_2).

[0012] In another embodiment, the invention is directed to hardened cements formed from such compositions.

[0013] The compositions and cements are advantageous in providing good radiopaque properties in combination with other properties which are advantageous for use in vivo, such as high strength, biocompatibility, osteoconductivity, and/or resorbability in vivo. These and additional advantages and embodiments of the invention will be more fully understood in view of the detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] Certain embodiments of the invention are set forth in the Examples and are described in connection with the Drawings, in which:

[0015] Fig. 1 presents radiography (72 kVp) results showing the relative radiopacity of 1 mm thickness calcium phosphate specimens containing 10% SrF_2 , $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$, SrBr_2 , and SrI_2 compared to commercial acrylic formulations, an aluminium standard, and trabecular bone, and showing that the specimens with SrBr_2 and SrI_2 have a higher radio-opacity than those with SrF_2 and $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$.

[0016] Fig. 2 presents relative radiopacity in mm Al for calcium phosphate specimens containing 10% SrF_2 , $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$, SrBr_2 , and SrI_2 compared to commercial acrylic formulations.

[0017] Fig. 3 presents radiography (72 kVp) results showing the relative radiopacity of 1 mm thickness calcium phosphate specimens containing 2, 10, and 20% SrBr_2 compared to commercial acrylic formulations, aluminum standard and trabecular bone, and showing that the addition of 10 wt% or more SrBr_2 gives a radioopaque cement.

[0018] Fig. 4 presents relative radiopacity in mm Al for calcium phosphate specimens containing 2, 10, and 20% SrBr_2 compared to commercial acrylic formulations.

[0019] Fig. 5 presents radiography (72 kVp) results showing the relative radiopacity of 1 mm thickness calcium phosphate specimens containing 2, 5, 10, 15, and 20% SrI_2 compared to commercial acrylic formulations, and an aluminum standard. Brushite specimens that contain between 10 and 20 wt% SrI_2 give a much higher radiopacity than all other specimens.

[0020] Fig. 6 presents relative radiopacity in mm Al for calcium phosphate specimens containing 2, 5, 10, 15, and 20% SrI_2 compared to commercial acrylic formulations.

[0021] Fig. 7 shows the compressive strength of calcium phosphate cements with 2, 10, and 20% of strontium halides. The dashed line represents the average value of the control composition.

[0022] Fig. 8 shows fluorescence microscopy of Saos-2 human osteoblast-like cells visualized by live/dead stain after 1 and 3 days in contact with calcium phosphate cements containing no strontium halide, 10% $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ and 10% SrF_2 (white: live cells; gray: dead cells).

[0023] Fig. 9 shows fluorescence microscopy of Saos-2 human osteoblast-like cells visualized by live/dead stain after 1 and 3 days in contact with calcium phosphate cements containing no strontium halide, 10% SrI_2 and 10% SrBr_2 (white: live cells; gray: dead cells).

[0024] Fig. 10 shows the relative radiopacity of calcium phosphate specimens calculated by image analysis with respect to an aluminum standard. The error bars correspond to the standard deviation. Commercial acrylic bone cements were included as controls. Different small letters represent statistically significant differences ($p < 0.05$) whereas equal small letters represent non-statistically significant differences ($p > 0.05$). (★) Simplex P was indistinguishable from the background under these particular conditions.

[0025] Fig. 11A shows compressive strength and Fig. 11B shows diametral tensile strength of calcium phosphate cements specimens that were kept for 24 h at 37°C in either air or PBS before testing. The error bars correspond to the standard deviation. Different small letters (air) or symbols (PBS) represent statistically significant differences ($p < 0.05$) whereas equal small letters (air) or symbols (PBS) represent non-statistically significant differences ($p > 0.05$).

[0026] Figs. 12A and 12B respectively show phase composition after 1 and 7 days conditioning in PBS at 37°C obtained from Rietveld refinement of the XRD data. The values that are not indicated correspond to values below 1.7 wt%.

[0027] Fig. 13 shows live/dead staining of Saos-2 cells cultured for 1 and 5 days on the different cement samples A) CP; B) CP- SrF_2 ; C) CP- SrCl_2 ; D) CP- SrBr_2 ; E) CP- SrI_2 . Live cells appear as white and dead cells as gray. No live cells were found on the 1-day culture on CP- SrF_2 .

[0028] Fig. 14A shows the total number of viable cells attached to the different cement samples after 1, 3 and 5 days. The error bars correspond to the standard deviation. No significant difference could be seen at any time point.

[0029] Fig. 14B shows the ALP activity of cells cultured on cement samples for 1, 3 and 5 days. No significant difference was observed between the groups.

[0030] Fig. 15 shows representative scanning electron microscopy micrographs of Saos-2 cells cultured on the different cement samples for 5 days for A) CP; B) CP-SrCl₂; C) CP-SrBr₂; D) CP-SrI₂.

[0031] The Drawings will be more fully understood in view of the detailed description and Examples.

DETAILED DESCRIPTION

[0032] The present invention relates to compositions suitable for use as, for example, dental, craniofacial or long-bone fillers, percutaneous vertebroplasty agents and kyphoplasty agents, as well as other implant type applications. The compositions comprise an injectable biomaterial cement and a radiopacity-improving agent in an amount sufficient to improve the radiopacity of the injectable biomaterial in vivo. The radiopacity agent comprises a water-soluble strontium halide, specifically strontium bromide (SrBr₂) and/or strontium iodide (SrI₂).

[0033] The injectable biomaterial cement may comprise any such cement known in the art, including, but not limited to acrylic cements and calcium-based cements such as calcium phosphate cements (CPC), calcium sulphate cements and calcium silicate cements. The compositions exhibit good radiopaque contrast and can be observed through computer tomography, radiography and/or fluoroscopy methods. In cases where the cement is resorbable, i.e., calcium phosphate cements, the strontium bromide and strontium iodide are particularly advantageous in that they are water-soluble. Accordingly, the material will slowly release strontium ions, which can also be beneficial for bone formation. The invention utilizes the same mixing procedure as standard cements, i.e., standard acrylic cements and standard calcium-based

cements such as calcium phosphate cements (CPC), calcium sulphate cements and calcium silicate cements, allowing surgeons to work with confidence and familiarity. In addition, the specific use of strontium iodide in calcium-based powder formulations, such as calcium phosphate powder formulations, for example, serves as an indicator of the exposure of the cement powder to air due to the oxidation of strontium and liberation of iodine, giving the cement powder a yellowish color.

[0034] The compositions of the present invention, employing the higher molecular mass strontium halides (SrBr_2 and SrI_2), have the following additional advantages in respect to SrCO_3 , SrF_2 , $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$:

- a) SrBr_2 and SrI_2 have a higher intrinsic radiopaque power due to their higher molecular mass, which would result in the use of lower amounts of radiopacifying agent required to give the same radiopacity as with SrCO_3 , SrF_2 , $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$.
- b) SrBr_2 and SrI_2 are also a source for Sr^{2+} release, which can be beneficial for bone formation.
- c) SrBr_2 and SrI_2 are water-soluble, unlike their closest radiopaque halide SrF_2 , which makes them ideal radiopacifying agents for resorbable biomaterials such as CPCs.
- d) SrBr_2 and SrI_2 are biocompatible and improve the cell viability of cells in contact with cements, for example, CPCs, that contain them. Additionally, they may improve the biocompatibility of bioinert materials such as acrylic bone cements.
- e) SrBr_2 and SrI_2 can act as porogen salts in non-resorbable formulations such as acrylic bone cements for vertebroplasty, to promote a porous structure and enhance the possibility for bone ingrowth.

- f) The Examples additionally show that the use of SrF_2 and $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ reduces the cell viability compared to their higher molecular mass halides containing bromine or iodine.

[0035] In one embodiment, the present invention relates a formulation of calcium-based cement such as calcium phosphate cement, calcium sulphate cement or calcium silicate cement, that includes one or more strontium halides, specifically strontium bromide and/or strontium iodide, as a radiopaque or radiopacity-improving agent. Calcium phosphate cements are used as bone fillers, commonly in dental and craniofacial applications. Despite their good osteoconductivity and ability to resorb *in vivo*, the use of calcium phosphate cements for applications, for example, in vertebroplasty, has, in the past, been limited due to the low intrinsic radiopacity of the cements. The compositions of the present invention overcome this disadvantage.

[0036] Calcium phosphate cements are typically based on a powder component and a liquid component. The powder component comprises a one or a mixture of several calcium orthophosphates, and the liquid component is an aqueous solution or a buffer. Upon mixing the two components, an injectable paste that can be administered through a cannula is formed, and, depending on the specific composition, one or more reactions occur and a solid material precipitates *in vivo*.

[0037] Calcium phosphate cements are basically classified into two types, depending on the final product of the setting reaction. The first type of calcium phosphate cements is apatite cement, in which the final product is hydroxyapatite (HA: $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), a material that is very similar to the mineral phase of bone. The second type of calcium phosphate cement is the brushite cement, in which the final product is dicalcium phosphate dihydrate (DCPD: $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$). Either of such types of calcium phosphate cements may be used in the

compositions of the present invention. The present invention incorporates the water-soluble radiopacity-improving agent, which may be dispersed in the powder component in the case of a powder/liquid system, or dissolved in a liquid in the case of a liquid/liquid system produced during formation of the cement. The radiopacity-improving agent is a strontium halide, specifically, strontium bromide (SrBr_2) and/or strontium iodide (SrI_2), which may be used alone or in combination.

[0038] The radiopacity-improving agent is used in an amount sufficient to improve the radiopacity of the injectable biomaterial cement in vivo. In specific embodiments, wherein the injectable biomaterial cement comprises an acrylic bone cement, the composition comprises not more than about 45 weight percent, based on the weight of the composition, of the radiopacity agent, or alternatively, comprises from about 1 to 45 weight percent, or more specifically from about 5 to 20 weight percent, based on the weight of the composition, of the radiopacity agent. In other embodiments, wherein the injectable biomaterial cement comprises a calcium phosphate cement-forming powder, the composition comprises from about 1 to 45 weight percent, based on the weight of the powder composition, of the radiopacity agent. In a more specific embodiment, the composition comprises from about 5 to 25 weight percent, based on the weight of the powder composition, of the radiopacity agent. In yet a more specific embodiment, the composition comprises from about 5 to 15 weight percent, based on the weight of the powder composition, of the radiopacity agent. The strontium halide may be added to the powder component simply by mixing in the desired formulation, depending on the type of cement that is desired.

[0039] The Examples show that the calcium phosphate cement compositions including the water-soluble radiopaque agents strontium bromide and/or strontium iodide result in additional advantages, including, but not limited to:

- a) SrBr_2 and SrI_2 improve the radiopacity of calcium phosphate cements.
- b) SrBr_2 and SrI_2 do not negatively affect the mechanical properties of calcium phosphate cements but on the contrary, improve them at certain concentrations.
- c) SrBr_2 and SrI_2 salts will dissociate in solution to slowly release Sr^{2+} ions, which may be beneficial for bone formation.
- d) SrBr_2 and SrI_2 do not only not adversely affect the viability of human osteoblast-like cells in contact with calcium phosphate cements that contain them but they also improve the viability after 3 days when compared to standard calcium phosphate or one containing SrF_2 or SrCl_2 .
- e) In the case of powder/liquid systems, the use of strontium iodide in calcium phosphate cement formulations is further advantageous because it can act as an indicator of the presence of air/humidity within the package of the powder component, as the oxidation of strontium liberates iodine, giving a yellowish color to the otherwise white powder.

[0040] The inclusion of SrBr_2 and SrI_2 , in calcium phosphate cements, and other degradable or non-degradable injectable biomaterial cements is a simple way to safely improve their radiopacity for visualization under computer tomography, X-ray, or real time fluoroscopy. In addition, the present Examples show that the inclusion of SrBr_2 and SrI_2 in calcium phosphate cements aids their strength and cell viability, providing a highly beneficial modification.

[0041] In a specific embodiment, for the preparation of the radiopaque calcium phosphate cement powder component, the desired calcium orthophosphate or mixture of calcium orthophosphates should be mixed with the desired amount of strontium halide.

[0042] In the case of a powder/liquid system, the powder component is prepared by blending together at least a calcium cement-forming powder, for example, a calcium

orthophosphate or mixture of calcium orthophosphates, in an amount of not less than about 55 weight percent powder, and a strontium halide (strontium bromide and/or strontium iodide), in an amount of not more than about 45 weight percent, based on the weight of the powder. The liquid component can be simply water or an aqueous solution, buffer, or ion-containing solution, although this list is not exhaustive. The powder and liquid may be used in any suitable ratio sufficient to form a hardened cement. In specific embodiments, a powder:liquid weight ratio of about 1:1 to about 10:1 is employed. In more specific embodiments, a powder:liquid weight ratio of about 1:1 to about 5:1 is employed. The powder component can be stored in a container which is resistant to air and humidity permeation, if desired.

[0043] For the preparation of a degradable radiopaque cement, the powder component is simply mixed with the liquid component. Preferably, the powder is added to the liquid. The components can be mixed manually or using any kind of mixing device and further transferred to a container, for example, a syringe/cannula system for injection into the bony defect or into a mould for prehardened implants.

[0044] The compositions of the present invention will provide enhanced performance to current applications of injectable biomaterial cements such as acrylic cements, calcium phosphate cements, calcium sulphate cements and calcium silicate cements, in various applications, including, but not limited to dental fillers, craniofacial fillers, percutaneous vertebroplasty, kyphoplasty, and long-bone fillers. Other applications within the scope of the invention will be apparent to those of ordinary skill in the implant field.

EXAMPLES

[0045] The following examples are provided to illustrate specific embodiments of the invention, without, however, limiting the scope of the invention defined by the claims. Both Examples 1 and 2 exemplify brushite cements containing different amounts of SrI_2 and SrBr_2 ,

respectively, compared to a standard brushite cement formulation (control), as well as formulations containing $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ or SrF_2 for mechanical testing and in vitro direct contact cell viability. The specimens for radiopacity were additionally compared to four commercial acrylic bone cements, namely, Osteopal V, Simplex P, Vertecem, and Vertecem V+, as well as a modified Simplex P including 10 or 30 wt% extra barium sulphate, as is known to be used in some cases.

Example 1

[0046] Brushite cements were prepared having compositions according to Table 1 by first dissolving monocalcium phosphate monohydrate (MCPM) and di-sodium dihydrogen pyrophosphate (1 wt %) in distilled water. Upon dissolution, a mixture of β -tricalcium phosphate (β -TCP) and strontium bromide (2, 10, and 20 wt%, respectively) were incorporated. The total powder-to-liquid ratio was 3.3 g/mL, and the molar ratio of MCPM: β -TCP was 1:1.

Table 1: Radiopaque brushite formulations containing SrBr_2

MCPM (wt %)	β-TCP (wt %)	SrBr_2 (wt %)	Pyrophosphate (wt %)	Powder/Liquid ratio
42.3	53	3.1	1.6	3.1
39.5	49.4	9.5	1.6	3.3
36.1	45.1	17.2	1.6	3.3

Example 2

[0047] Brushite cements were prepared having compositions according to Table 2 by first dissolving monocalcium phosphate monohydrate (MCPM) and disodium dihydrogen pyrophosphate (1 wt%) in distilled water. Upon dissolution, a mixture of β -tricalcium phosphate (β -TCP) and strontium iodide (2, 5, 10, 15, and 20 wt%, respectively) were incorporated. The total powder-to-liquid ratio was 3.3 g/mL, and the molar ratio MCPM: β -TCP was 1:1.

Table 2: Radiopaque brushite formulations containing SrI₂

MCPM (g)	β-TCP (g)	SrI₂ (g)	Pyrophosphate (g)	Water (mL)
5.00	6.15	0.22	0.11	3.45
5.00	6.15	0.56	0.12	3.55
5.00	6.15	1.12	0.12	3.72
5.00	6.15	1.67	0.13	3.89
5.00	6.15	2.23	0.13	4.06

[0048] The preparation and testing methods were the same for both Examples 1 and 2 and will be explained next. Upon mixing the liquid and the powder components for 30 seconds, specimens were molded for use in radiopacity studies, compression testing, and in vitro direct contact viability assays.

Radiopacity

[0049] For the radiopacity tests, discs of 1 mm height and 10 mm diameter were molded. The specimens were irradiated at 72 kV_p and the radiopacity was calculated relative to an aluminum ladder (1 to 5 mm) by determining the relative amount of light and generating a standard curve.

[0050] Figs. 1 and 2 show the relative radiopacity of brushite cements containing 10 wt% SrBr₂ and SrI₂, respectively, compared to standard brushite cements, brushite cements containing 10 wt% SrCl₂·6H₂O, and SrF₂, and commercial bone cements. A sample of trabecular bone from the knee is also shown in Fig. 1 for observation only, since this sample is not of standard thickness. Fig. 2 shows that brushite cements containing 10 wt% SrI₂ are more radiopaque than all the other specimens, except Osteopal V, which contains 45 wt% ZrO₂; however, their radiopacities are comparable as seen in Fig. 1.

[0051] Figs. 3 and 4 show the relative radiopacity of some of the brushite cements from Example 2, which contain 2, 10, and 20 wt% SrBr₂, respectively, compared to standard brushite

and commercial bone cements. It is observed that brushite specimens that contain 20 wt% SrBr_2 give similar radiopacity to most acrylic cements, even those containing up to 45 wt% ZrO_2 .

[0052] Figs. 5 and 6 show the relative radiopacity of brushite cements from Example 1, which contain 2, 5, 10, and 20 wt% SrI_2 , respectively, compared to standard brushite and commercial bone cements. It is observed that brushite specimens that contain between 10 and 20 wt% SrI_2 give a much higher radiopacity than all other specimens.

Compressive strength

[0053] The specimens for the compression tests were standard 6 mm diameter and 12 mm height cylinders that were molded in rubber. The specimens were stored at 37°C, and tested after 24 h using an AGS-H universal materials testing machine at a crosshead displacement rate of 1 mm/min. The compressive strength was obtained from the load-versus-displacement curves and is listed in Table 3. Table 3 indicates that, in general, calcium phosphate cements containing strontium halides have a higher strength than the standard material without strontium halide. In addition, Fig. 7 shows that 10 wt% seems to be an optimal strontium halide concentration in terms of mechanical strength. Moreover, the respective formulations containing 10 wt% SrBr_2 and SrI_2 exhibited the highest values of strength, reaching 8.67 ± 1.86 MPa and 7.80 ± 1.01 MPa, respectively. These results indicate that the compositions of this invention not only show a better radiopacity than most commercial radiopaque materials but also a small improvement in mechanical properties with respect to standard calcium phosphate cements and other calcium phosphate containing $\text{SrF}_2/\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$, which are used in commercial cements.

Table 3. Compressive strength of different calcium phosphate cement compositions including strontium halides

Example	Type of calcium phosphate cement	% Strontium halide	Number of specimens	Compressive strength (MPa $\pm$ SD)
-	Control	0	16	6.63 ± 0.99

-	SrF ₂	2	16	6.12 ± 0.89
-	SrF ₂	5	15	6.97 ± 0.82
-	SrF ₂	10	16	6.75 ± 0.93
-	SrF ₂	15	16	6.39 ± 0.84
-	SrF ₂	20	15	6.62 ± 0.99
-	SrCl ₂ ·6H ₂ O	2	16	5.45 ± 0.69
-	SrCl ₂ ·6H ₂ O	5	16	6.30 ± 0.97
-	SrCl ₂ ·6H ₂ O	10	15	7.21 ± 0.80
-	SrCl ₂ ·6H ₂ O	15	15	7.39 ± 0.77
-	SrCl ₂ ·6H ₂ O	20	15	6.54 ± 0.99
Example 1	SrBr ₂	2	10	7.42 ± 1.34
Example 1	SrBr ₂	10	8	8.67 ± 1.86
Example 1	SrBr ₂	20	7	1.69 ± 0.22
Example 2	SrI ₂	2	16	6.73 ± 0.93
Example 2	SrI ₂	5	16	6.85 ± 0.65
Example 2	SrI ₂	10	16	7.80 ± 1.01
Example 2	SrI ₂	15	8	6.75 ± 1.03
Example 2	SrI ₂	20	11	3.46 ± 1.54

Direct contact in vitro cell viability test

[0054] For the in vitro viability tests, specimens containing 10 wt% SrCl₂·6H₂O, SrF₂, SrBr₂, and SrI₂, respectively, as well as a control specimen, were molded in rubber molds and soaked in PBS for 18h. The samples were then sterilized by UV radiation during 1 h (30 min per side). The cells (human osteosarcoma cell line Saos-2) were seeded at a cell density of 15,000 cells/cm² on the samples. The medium was DMEM F12 supplemented with 10% FBS, streptomycin/penicillin/glutamine.

[0055] After 24 and 72 h, the samples were stained with live/dead stain and observed under the fluorescent microscope. New specimens were used at each time point. The results are

shown in Figs. 8 and 9. Fig. 8 shows the fluorescence images for the control and the brushite specimens containing 10 wt% $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ and SrF_2 . It is observed that in the case of the $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$, all cells died after 3 days, whereas on the specimens containing SrF_2 , the cells died already after 1 day, compared to the control where live cells are observed.

[0056] Fig. 9 shows the fluorescence images for the control and the respective brushite specimens containing 10 wt% SrBr_2 and SrI_2 . These very interesting results show the advantage of the radiopaque cements including SrBr_2 and SrI_2 , since the cell viability not only was not affected, but was improved after 3 days compared to the control. This is accounted for by the higher number of white dots in the image. The gray dots are absorbed in the black parts.

Example 3

[0057] In this Example, cements prepared from compositions according to the present invention were compared with various comparative and commercial products containing conventional additives. All reagents were acquired from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA) unless otherwise specified. Compositions according to the invention were prepared using a powder-to-liquid ratio of 3.3 g/mL and an MCPM; β -TCP molar ratio of 1:1. Firstly, appropriate amounts of MCPM (purum p.a., $\geq 85\%$, KT) and disodium dihydrogen pyrophosphate (1 wt% of the total amount of powder) were mixed with distilled water to form a slurry. Secondly, appropriate amounts of β -TCP (purum p.a., $\geq 96\%$, calc. as $\text{Ca}_3(\text{PO}_4)_2$, KT) and SrX (10 wt% of the total mass), previously mixed with each other, were added to the slurry and mixed with a metal spatula for 30 seconds. The following SrXs were tested: strontium fluoride, strontium chloride hexahydrate ($\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$), strontium bromide, and strontium iodide. For simplicity, the materials were labeled as follows: unmodified brushite cement (CP), brushite

cement containing SrF_2 (CP- SrF_2), brushite cement containing $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ (CP- SrCl_2), brushite cement containing SrBr_2 (CP- SrBr_2), and brushite cement containing SrI_2 (CP- SrI_2).

Radiopacity

[0058] To measure the radiopacity, nine groups consisting of six disc-shaped specimens ($\varnothing=10$ mm; $h=1$ mm) were prepared. The specimens were placed on an X-ray film cassette and irradiated inside an X-ray generator (Faxitron Bioptics, Tucson, AZ, USA) at a peak kilovoltage of 72 kV_p. Radiographs were developed and the radiopacity (in mm Al) was calculated by generating a standard curve (amount of light vs. mm Al) using an aluminum scale (scaled 1 to 5 mm). The amount of light (scaled 0 to 255) was determined using the curves feature under the adjustments tool in Adobe Photoshop CS4 software (Adobe Systems Incorporated, San José, CA, USA). Commercial acrylic bone cements Simplex® P, 10 wt% BaSO_4 (Stryker Corporation, Kalamazoo, MI, USA), Osteopal® V, 45 wt% ZrO_2 (Heraeus GmbH, Hanau, Germany), Vertecem®, 30 wt% BaSO_4 and Vertecem® V+, 40 wt% ZrO_2 (Synthes, Solothurn, Switzerland), were used as controls. Simplex® P is indicated for e.g. prosthesis fixation, while Osteopal® V, Vertecem® and Vertecem® V+ are indicated for vertebroplasty.

[0059] The relative radiopacity of the brushite cements was found to increase with an increase in the atomic number of the halogen, as shown in Fig. 10. The specimens containing strontium fluoride had a similar radiopacity to control brushite cement whereas the specimens containing strontium chloride had a radiopacity closer to that of specimens containing strontium bromide. The specimens containing strontium bromide and strontium iodide were significantly more radiopaque than control, strontium fluoride-, and strontium chloride-containing brushite specimens, and not statistically different from Vertecem® V+.

Mechanical testing

[0060] To measure the compressive strength (σ_{CS}), cylindrical specimens of 12 mm height and 6 mm diameter were prepared (ASTM-F451, Standard Specification for Acrylic Bone Cement (2008)). To measure the diametral tensile strength (σ_{DTS}), disc-shaped specimens ($\varnothing=8$ mm, $h=3.3$ mm) were prepared. All specimens were tested to failure using an AGS-H universal materials testing machine (Shimadzu, Kyoto, Japan) by applying a uniaxial compressive load at a crosshead displacement rate of 1 mm/min. To assess the effect of cement and radiopacifier dissolution at physiological conditions, one group of specimens was stored for 24 h in phosphate buffered saline (PBS) at 37°C whereas another group was stored for 24 h in air at 37°C. The compressive strength and break force (F_{max}) were obtained from the load-versus-displacement curves. The diametral tensile strength was calculated according to equation 2 (New American Dental Association Specification No. 27, direct filling resins (1977)), where D is the diameter, and H is the height of the specimen.

$$\sigma_{DTS} = 2F_{max} / \pi \cdot D \cdot H \quad (2)$$

[0061] Fig. 11A shows the σ_{CS} and Fig. 11B shows the σ_{DTS} of the different bone cement groups. The PBS set groups had, in general, lower σ_{CS} and σ_{DTS} than their air set counterparts. The σ_{CS} varied from 4.94 ± 0.64 (CP-SrCl₂) to 6.55 ± 1.16 MPa (CP-SrBr₂) for the air set group, and from 2.13 ± 0.21 (CP-SrCl₂) to 5.74 ± 1.14 MPa (CP) for the PBS set group. The groups containing strontium chloride had, in general, the lowest σ_{CS} and σ_{DTS} of all groups. Air set groups containing strontium bromide and strontium iodide had a higher σ_{CS} than the other specimens, although no statistically significant differences were found with respect to e.g. the control group. On the other hand, PBS set groups containing strontium bromide or strontium iodide had a statistically significant lower σ_{CS} than the control group. In addition,

σ DTS varied from 1.55 ± 0.16 (CP-SrCl₂) to 3.65 ± 0.30 MPa (CP) for the air set specimens, and from 1.14 ± 0.18 (CP-SrCl₂) to 1.88 ± 0.24 MPa (CP) for the PBS set groups. The air set control group had a statistically significant higher σ DTS than all the other groups, whereas the groups containing strontium fluoride, strontium bromide and strontium iodide had, in general, all similar σ DTS values. Furthermore, when the specimens were set in PBS, the control and the group containing strontium bromide had, in general, the highest σ DTS, followed by strontium iodide, strontium fluoride, and strontium chloride.

X-ray diffraction

[0062] The phase analysis of the different cements was performed by X-ray diffraction (XRD) on a D8 Advance diffractometer (Bruker Corporation, Billerica, MA, USA). The cements were prepared as described above and stored in PBS at 37°C for 1 or 7 days. The specimens were thoroughly ground using a glass pestle and mortar prior to XRD analysis. The data was acquired with Cu K α radiation (40 kV, 40 mA) in the 2θ interval between 5 and 60°, with a step size of 0.0143°, and a count time of 5 s. The phase composition and lattice parameters in the resulting phases were obtained from Rietveld refinement using BGMN 4.2.20 software (Dr. J. Bergmann, Ludwig-Renn-Allee 14, D-01217 Dresden, Germany) and the corresponding powder diffraction files for the different phases.

[0063] Figs. 12A and 12B show the phase composition of the different bone cement groups conditioned in PBS for 1 and 7 days as determined from Rietveld refinement. The cements were predominantly composed of brushite and a fraction of unreacted β -calcium pyrophosphate (β -CPP, an impurity present in the β -TCP) and β -TCP. The SrF₂, unlike the other strontium halides, remained unreacted, and was present in the CP-SrF₂ group. The CP-SrBr₂ and

CP-SrI₂ groups also had significant amounts of monetite, which was higher in the groups that were stored in PBS for 7 days. All other phases were present in amounts of ≤ 1.7 wt%.

[0064] Table 4 shows the volume of the brushite and monetite crystals, as calculated from the lattice parameters, for a monoclinic and a triclinic system, respectively. The lattice parameters decreased, in both brushite and monetite, with the time that the specimens were immersed in PBS at 37°C. Additionally, the lattice parameters were higher for CP-SrCl₂, CP-SrBr₂, and CP-SrI₂ than CP and CP-SrF₂. The crystals were always slightly smaller after 7 days storage in PBS except for brushite crystals in the CP-SrI₂ group. The size of the brushite crystals decreased in the order CP-SrBr₂ > CP-SrCl₂ > CP-SrI₂ > CP > CP-SrF₂. Furthermore, the size of the monetite crystals decreased in the order CP-SrBr₂ > CP-SrI₂. The brushite crystals in CP-SrCl₂, CP-SrBr₂, and CP-SrI₂ groups, were larger than those in CP and CP-SrF₂ groups.

Table 4: The volume of brushite and monetite crystals as calculated from the lattice parameters for a monoclinic and a triclinic system, respectively, after 1 and 7 days treatment in PBS at 37°C.

Cement	Phase	Days in PBS	Lattice parameters						V [Å ³]
			a [Å]	b [Å]	c [Å]	α [°]	β [°]	γ [°]	
CP	brushite	1	5.81581	15.19889	6.24785	-	116.370	-	494.81
	brushite	7	5.81571	15.19821	6.24751	-	116.377	-	494.72
CP-SrF ₂	brushite	1	5.81464	15.19693	6.24714	-	116.371	-	494.58
	brushite	7	5.81381	15.19483	6.24562	-	116.377	-	494.30
CP-SrCl ₂	brushite	1	5.82110	15.21450	6.26016	-	116.348	-	496.84
	brushite	7	5.81897	15.21037	6.25732	-	116.359	-	496.25
CP-SrBr ₂	brushite	1	5.82126	15.21544	6.26072	-	116.348	-	496.93
	brushite	7	5.82019	15.21268	6.25916	-	116.360	-	496.57
CP-SrI ₂	monetite	1	6.89660	6.81400	7.01530	96.760	103.128	89.027	302.05
	monetite	7	6.14950	6.64600	7.01114	96.287	103.830	88.522	299.77
	brushite	1	5.81975	15.21160	6.25802	-	116.352	-	496.44
	brushite	7	5.81838	15.20816	6.25530	-	116.363	-	495.95
	monetite	1	6.91129	6.45800	7.00849	96.334	103.830	88.514	299.43
	monetite	7	6.91237	6.64538	7.00790	96.303	103.889	88.473	299.31

In vitro studies with Saos-2 cells

[0065] For the cell studies, disc-shaped specimens ($\varnothing=13$ mm, $h=2$ mm) were prepared. The specimens were set in air for 2 hours and stored in PBS at 37°C for 24 h. The specimens were then UV sterilized for 45 min on each side and put in 24-well plates. Saos-2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT, USA) and 2 mM L-glutamine, 100 U/mL penicillin and 0.1 mg/mL streptomycin and kept at 37°C in a humidified atmosphere with 5% CO₂. Cells were seeded on the specimens at a density of 50,000 cells/cm² and cultured for 1, 3 and 5 days. The medium was replaced with fresh medium every day. All the assays were performed in two independent experiments with 4 replicates for each group.

Live/dead stain

[0066] The number of viable cells was visualised after 24 h and 5 days using live/dead stain kit (Life Technologies, Carlsbad, CA, USA). Briefly, the specimens were rinsed in PBS twice followed by 15 min incubation in staining reagents according to the manufacturer's protocol. The cells were then visualized using a fluorescent microscope (Carl Zeiss AG, Oberkochen, Germany) and representative micrographs were acquired in which live cells fluoresced green and dead cells fluoresced red.

[0067] Fig. 13 shows that fluorescent staining of live and dead cells on cement samples revealed some differences between groups. On samples corresponding to the CP-SrF₂ group there were no cells alive after 1 day (2 repetitions in quadruplicate wells); therefore, this group was excluded from further cell studies. The rest of the groups resulted in similar cell morphology and amount of viable cells with respect to control brushite.

Cell proliferation and alkaline phosphatase (ALP) activity

[0068] The total number of cells attached to cement specimens was assessed by measuring the activity of lactate dehydrogenase (LDH) enzyme, using a TOX 7 kit. The specimens were then transferred to a new 24-well plate after which the cells were lysed and subsequently incubated with the LDH reagents for 20 min. The colour change was measured at 560 nm and 620 nm with a microplate reader (Tecan, Männedorf, Switzerland).

[0069] The osteogenic differentiation was determined by measuring the ALP activity, which is an early osteogenic marker. Yellow Liquid Substrate Systems for ELISA were added to aliquots of the cell lysis, prepared as described in cell proliferation section, and incubated for 5 min. The reaction was stopped with 3M NaOH and the colour change was measured at 405 nm with a microplate reader. The ALP values were normalized to the total number of cells according to the LDH assay and expressed as p-nitrophenyl pmols/min/1000 cells. Standard ALP curves were made with p-nitrophenyl phosphate.

[0070] Figs. 14A and 14B show that no significant differences in cell number between the samples could be detected. However the proliferation followed the same trend in all cases; the amount of cells was similar at day 1 and 3 and increased slightly until day 5. No significant differences in ALP activity were revealed between the different groups. A similar trend was observed for all groups; the ALP activity increased slightly at day 3 and remained at a similar level until day 5.

Scanning electron microscopy

[0071] The morphology of the Saos-2 cells cultured on the cement specimens as well as the cement microstructures were visualised by scanning electron microscopy (SEM, LEO 1550, Carl Zeiss AG, Oberkochen, Germany). After 5 days of culture, selected cement specimens from

each group were rinsed twice in PBS and fixed with 2.5 % glutaraldehyde/PBS solution for 60 min at 4°C. Samples were subsequently rinsed twice in PBS and dehydrated in several steps with graded ethanol followed by further dehydration in hexamethylsilazane. The specimens were sputter-coated with palladium prior to SEM imaging. The images were acquired at 2.00 kV using a secondary electron detector for topographic contrast.

[0072] The SEM micrographs (Fig. 15) showed that the cells cultivated on different cement samples all exhibited similar flattened morphology. Moreover, all the cements exhibited microstructures consisting of interlocked plate-like structures with varying sizes.

[0073] The examples and specific embodiments set forth herein are illustrative in nature only and are not to be taken as limiting the scope of the invention defined by the following claims. Additional specific embodiments and advantages of the present invention will be apparent from the present disclosure and are within the scope of the claimed invention.

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What is claimed is:

1. A composition comprising an injectable biomaterial cement and a radiopacity-improving agent in an amount sufficient to improve the radiopacity of the injectable biomaterial cement *in vivo*, wherein the radiopacity agent comprises strontium bromide (SrBr_2) and/or strontium iodide (SrI_2).
2. The composition of claim 1, wherein the injectable biomaterial cement comprises an acrylic bone cement and the composition comprises not more than about 45 weight percent, based on the weight of the composition, of the radiopacity agent.
3. The composition of claim 1, wherein the injectable biomaterial cement comprises a resorable calcium phosphate cement-forming powder.
4. The composition of claim 3, wherein the calcium phosphate cement-forming powder comprises a hydroxyapatite cement-forming powder.
5. The composition of claim 3, wherein the calcium phosphate-forming powder comprises a brushite cement-forming powder.
6. The composition of claim 5, wherein the brushite cement-forming powder comprises monocalcium phosphate monohydrate and β -tricalcium phosphate.

7. The composition of claim 1, wherein the injectable biomaterial cement comprises a calcium sulphate cement-forming powder or a calcium silicate cement-forming powder.
8. The composition of any one of claims 3-7, wherein the composition comprises from about 1 to 45 weight percent, based on the weight of the cement-forming powder, of the radiopacity agent.
9. The composition of any one of claims 3-7, wherein the composition comprises from about 5 to 25 weight percent, based on the weight of the composition, of the radiopacity agent.
10. The composition of any one of claims 3-9, wherein the injectable biomaterial cement further comprises an aqueous liquid.
11. The composition of claim 10, wherein the liquid comprises a buffer.
12. The composition of any one of claims 3-11, provided in a container which is resistant to air and humidity permeation.
13. The composition of any one of claims 1-12, wherein the radiopacity agent comprises strontium bromide (SrBr_2).
14. The composition of any one of claims 1-12, wherein the radiopacity agent comprises strontium iodide (SrI_2).

15. A hardened cement formed from the composition of any one of claims 1-14.

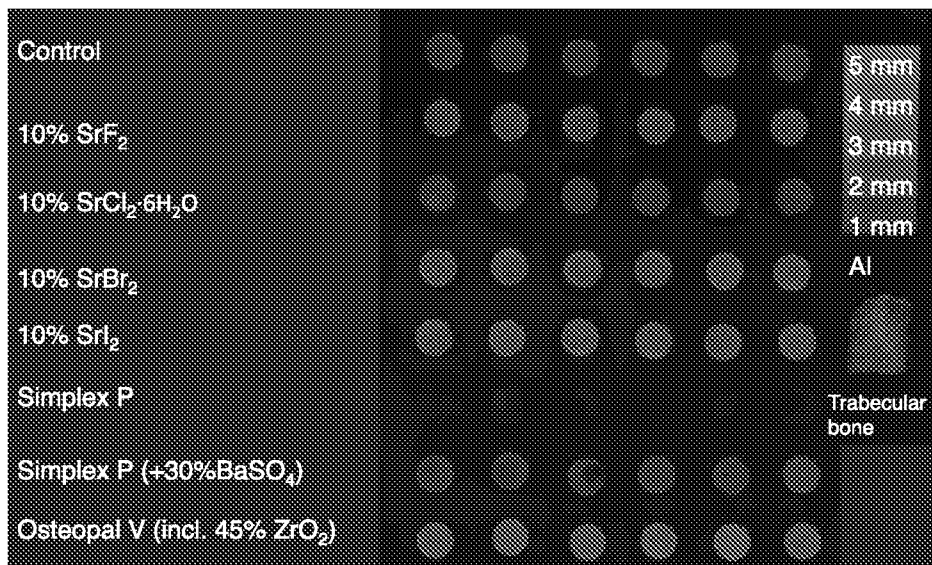


FIG. 1

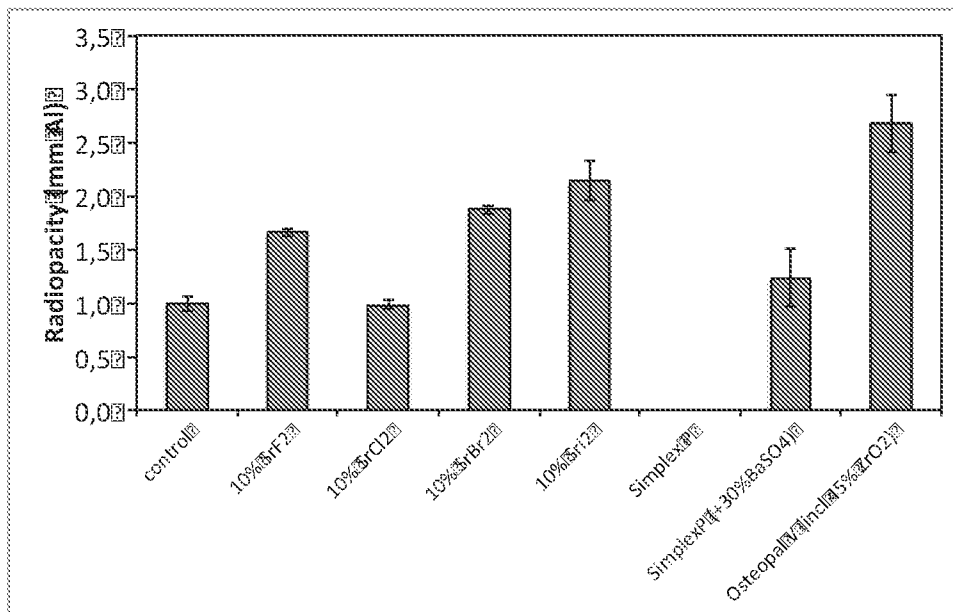


FIG. 2

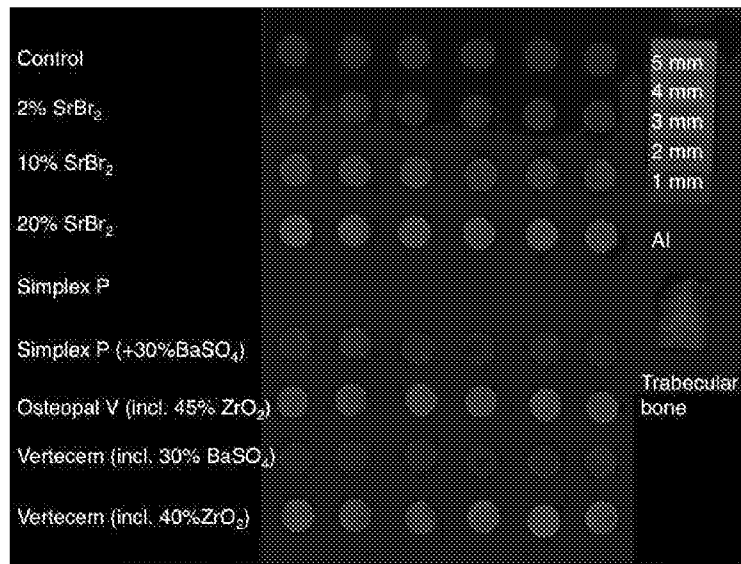


FIG. 3

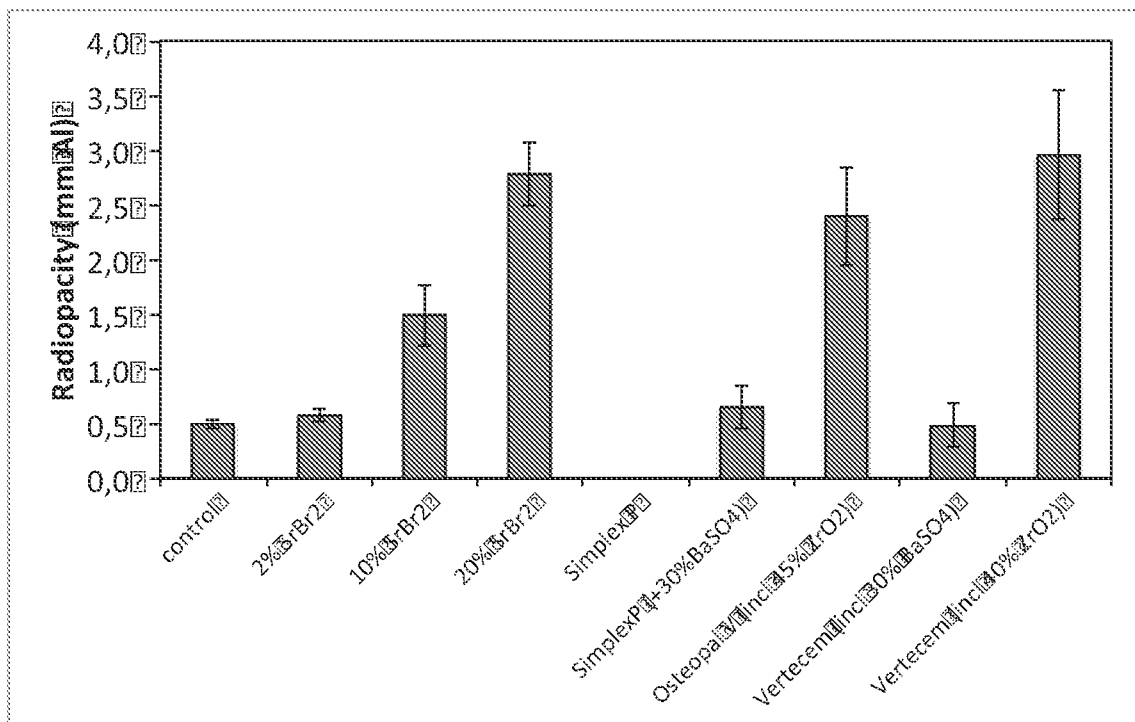


FIG. 4

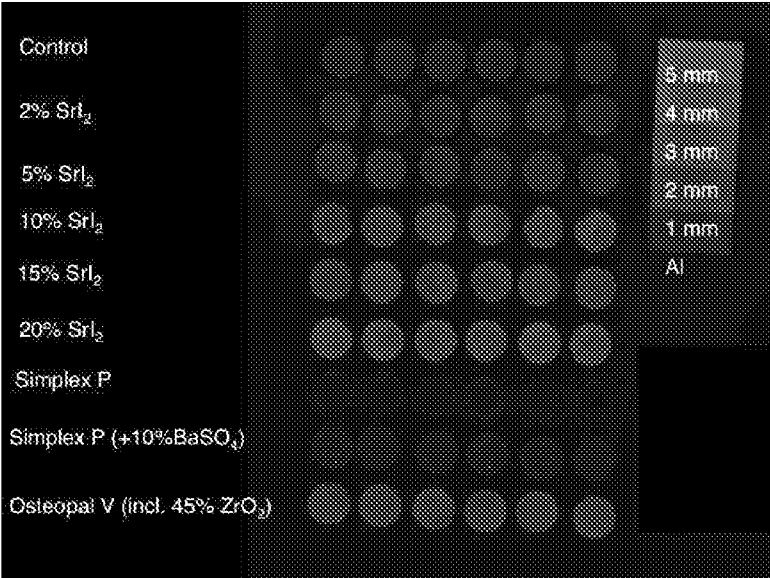


FIG. 5

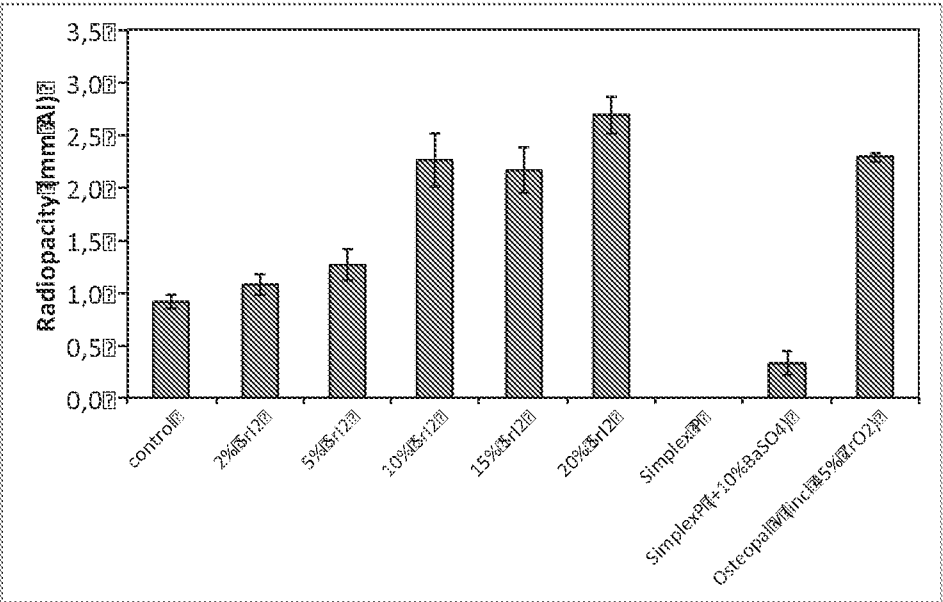


FIG. 6

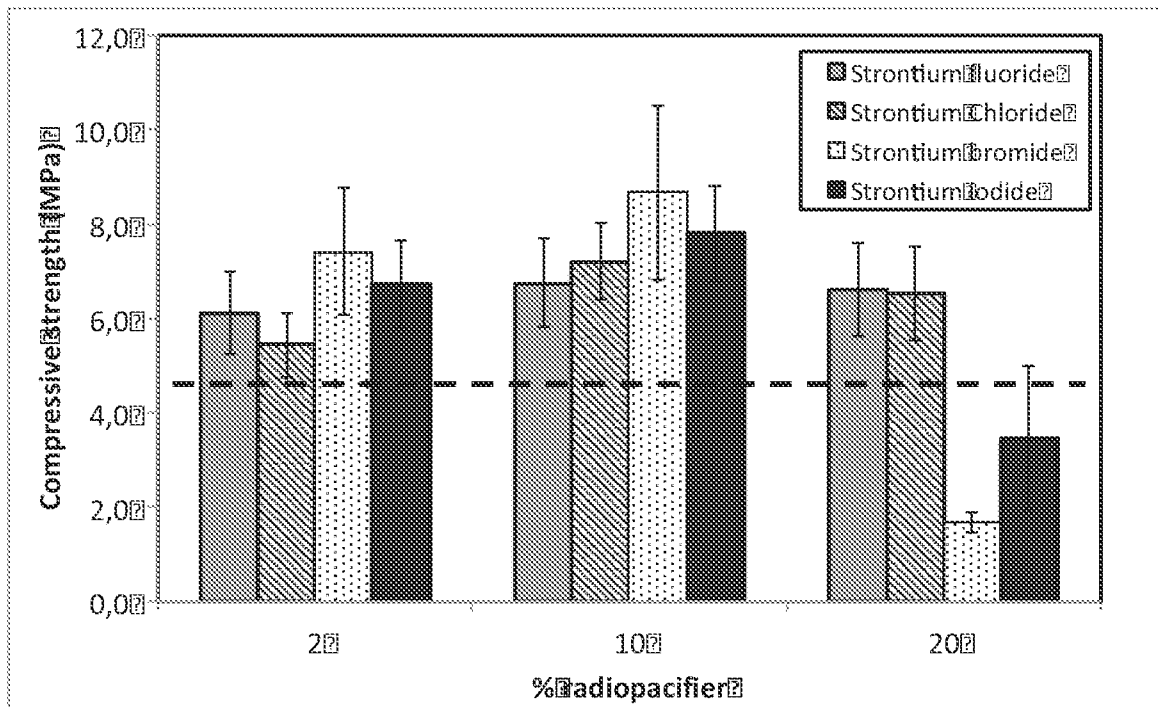


FIG. 7

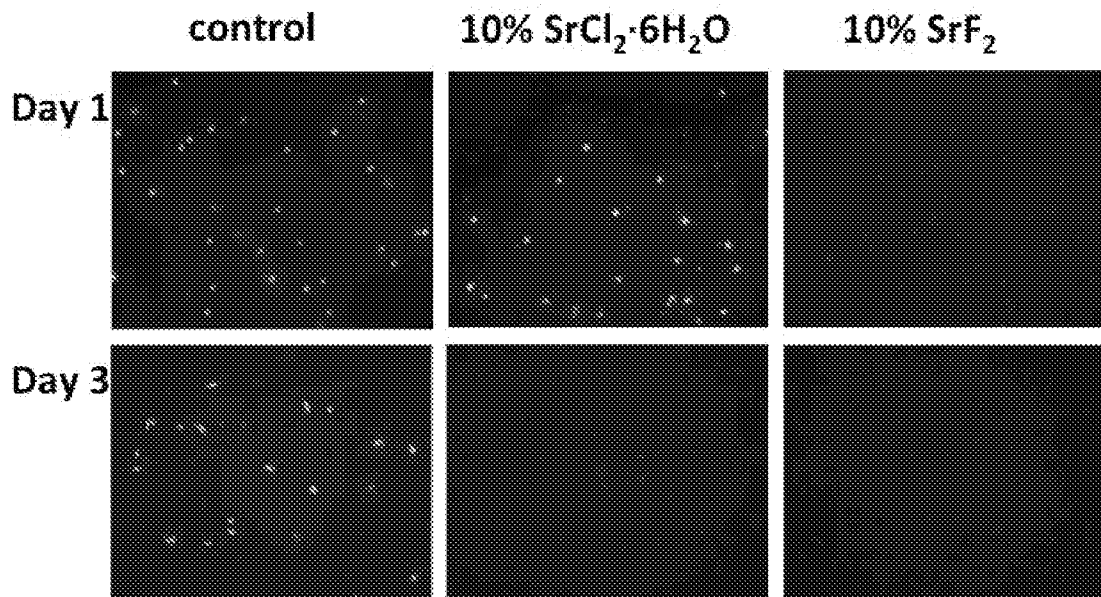


FIG. 8

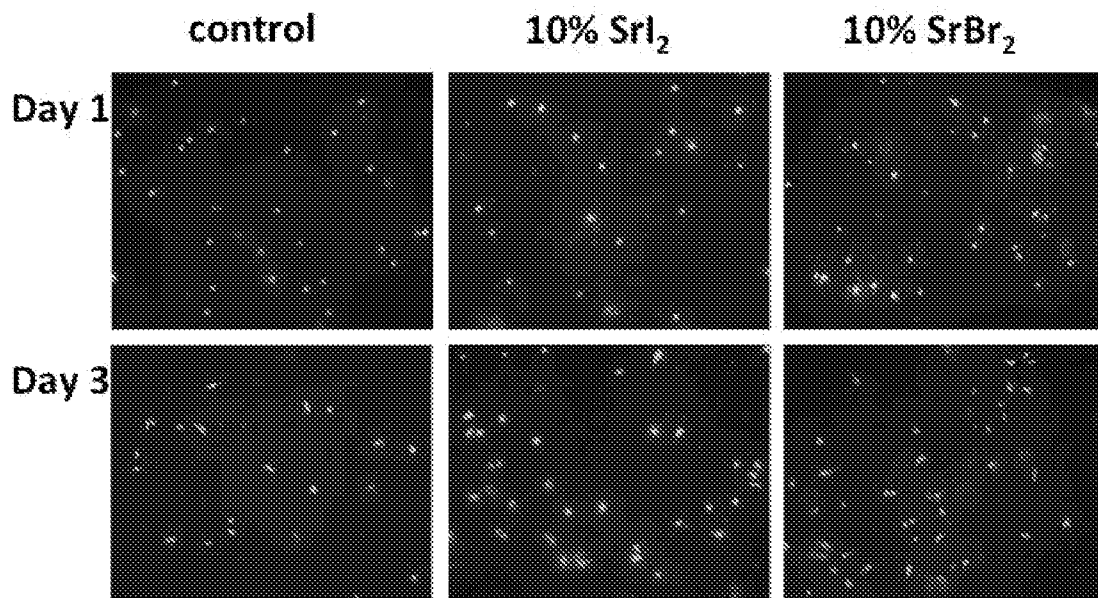


FIG. 9

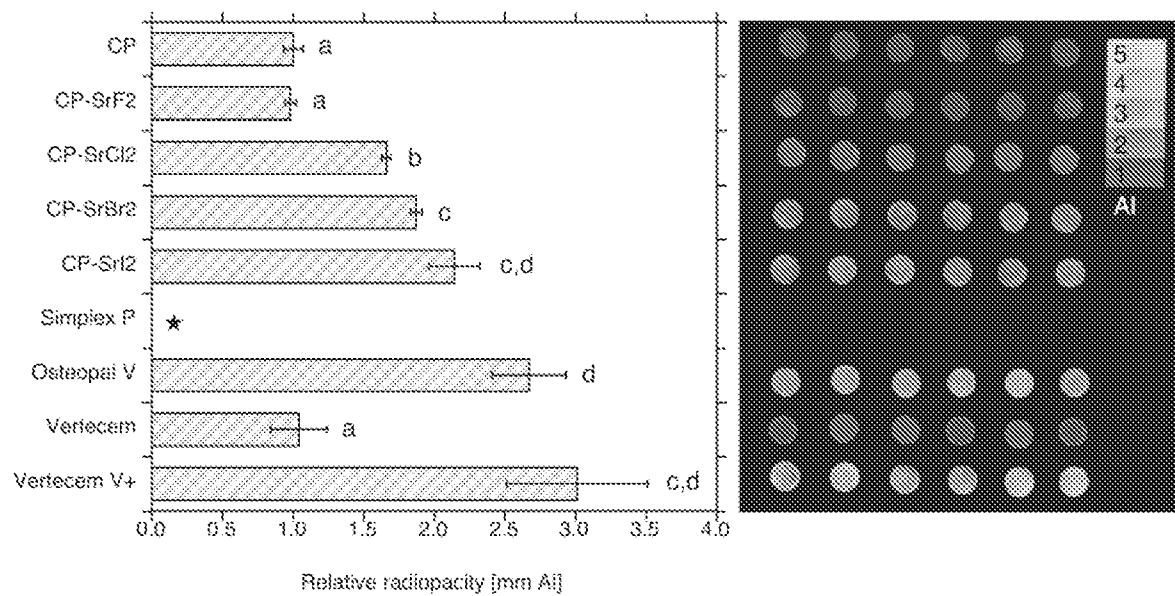


FIG. 10

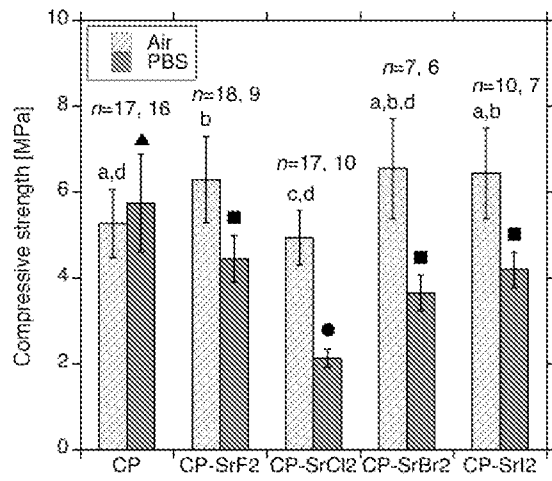


FIG. 11A

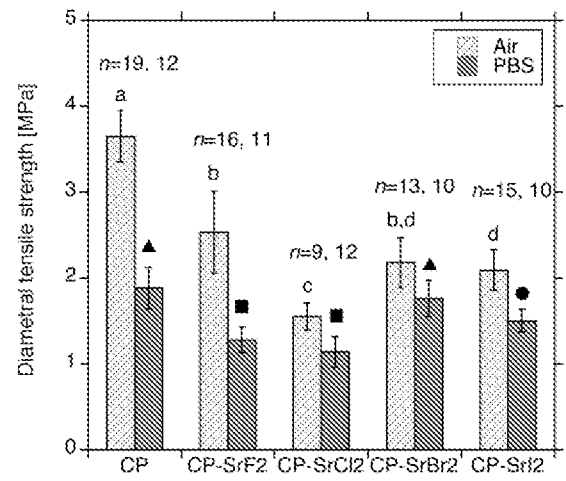


FIG. 11B

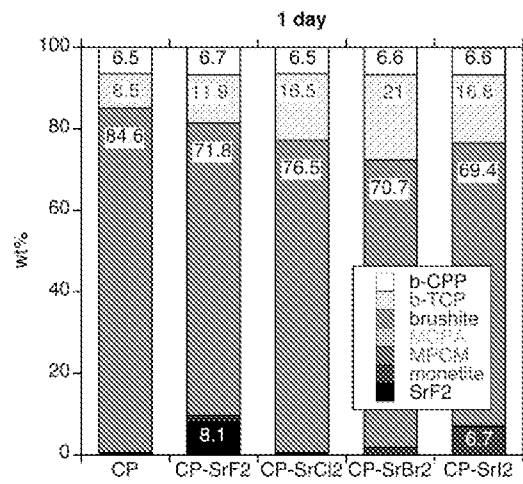


FIG. 12A

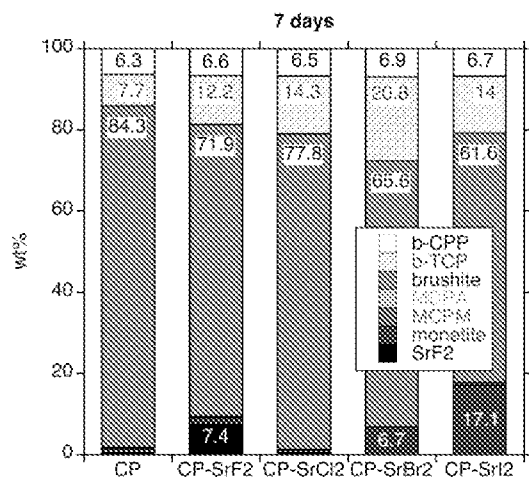


FIG. 12B

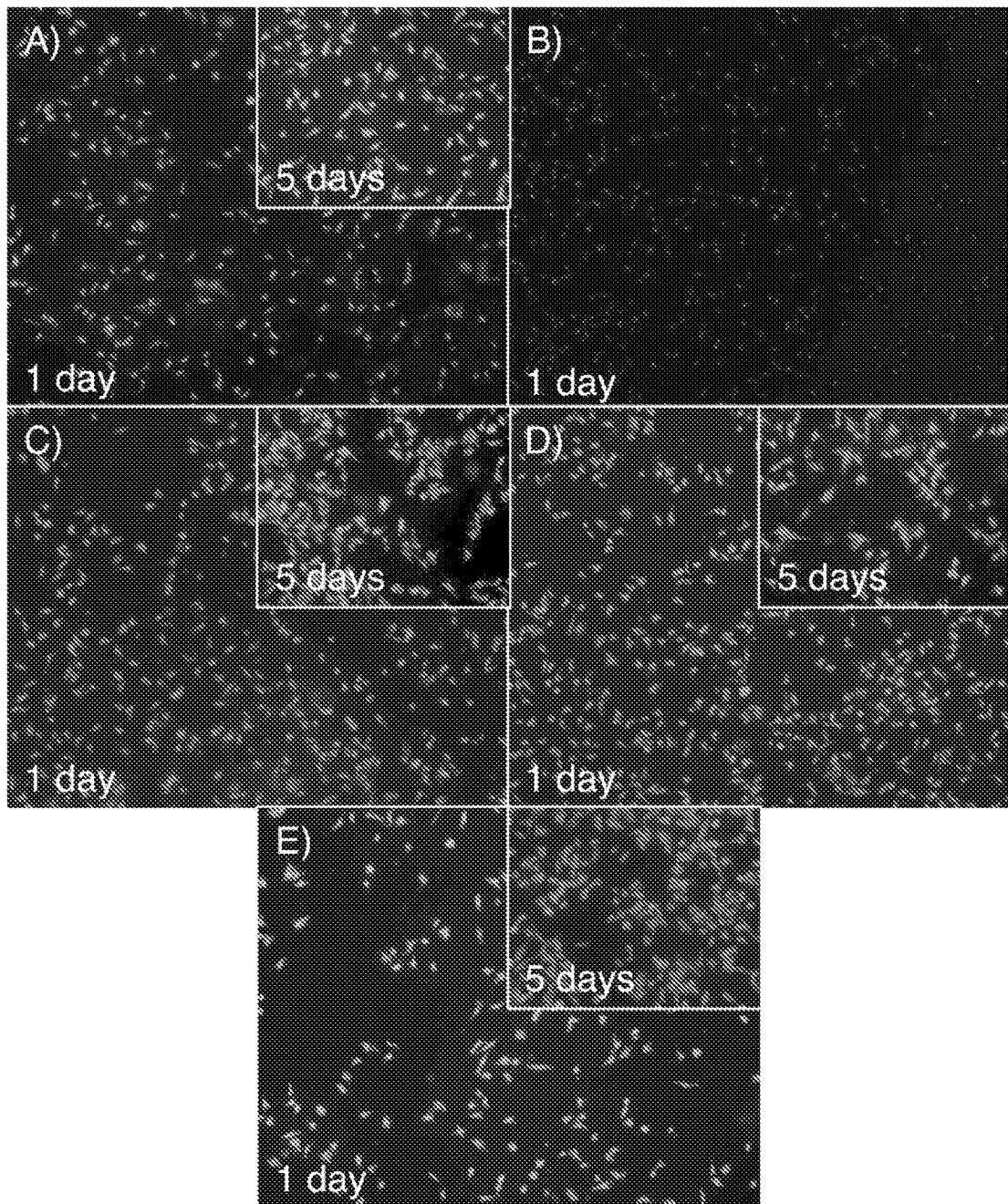


FIG. 13

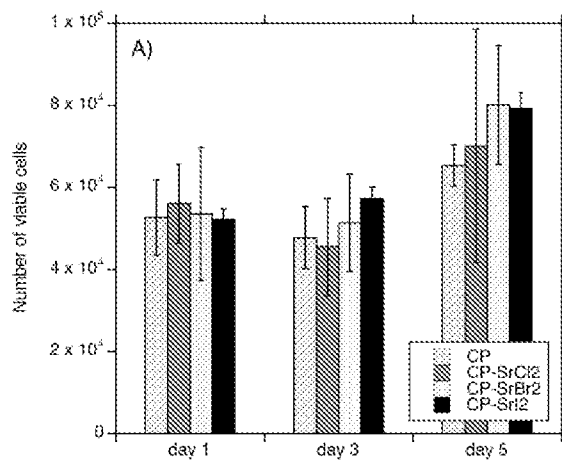


FIG. 14A

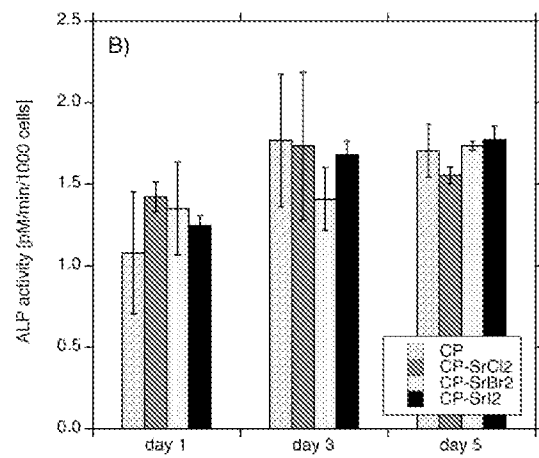


FIG. 14B

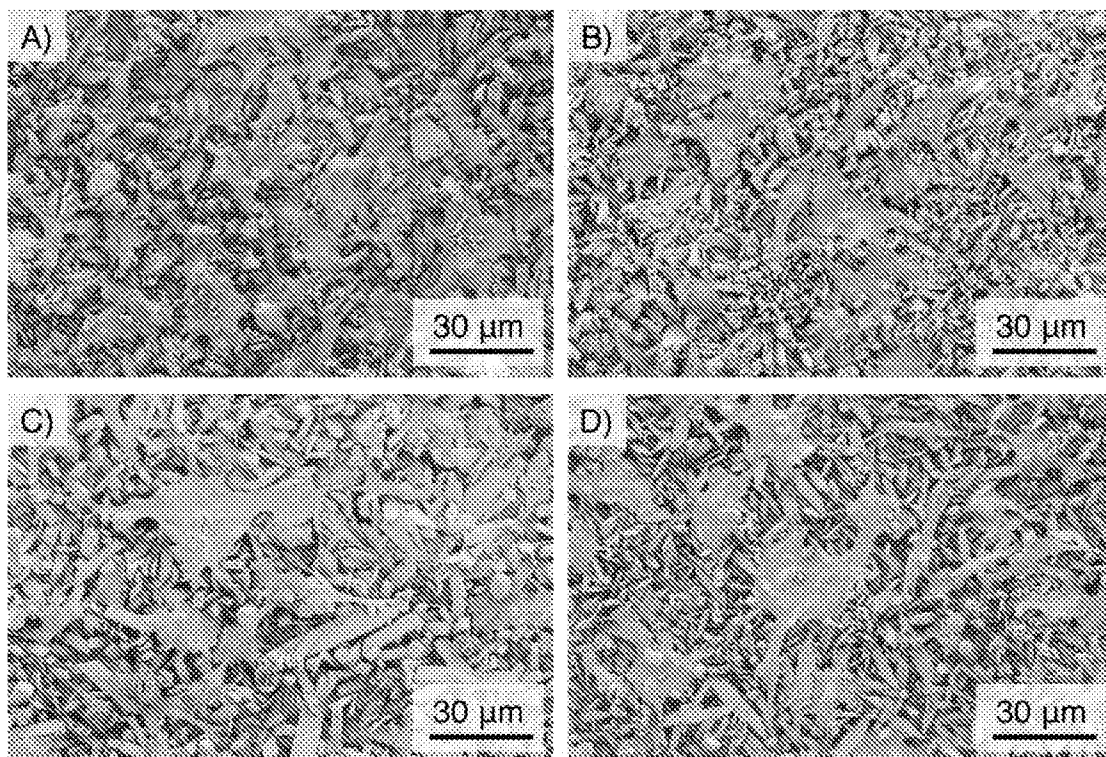


FIG. 15